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Ping-Chi Hsu [a], Te-Jen Lai [b], Nai-Wen Guo [c], George H. Lambert [d] & Yueliang Leon Guo [e]

[a] Department of Safety, Health and Environmental Engineering, National Kaohsiung First University of Science and Technology, Kaohsiung, Taiwan

[b] Department of Psychiatry, Chung-Shan Medical University Hospital, Taichung, Taiwan

[c] Department of Psychology, Kaohsiung Medical University, Kaohsiung, Taiwan

[d] NTH/USEPA Center for Childhood Neurotoxicology and Exposure Assessment and the Department of Pediatrics, University of Medicine and Dentistry of New Jersey and Rutgers University, One Robert Wood Johnson Place, New Brunswick, NJ, USA

[e] Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan

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SERUM HORMONES IN BOYS PRENATALLY EXPOSED TO POLYCHLORINATED BIPHENYLS AND DIBENZOFURANS

Ping-Chi Hsu[1], Te-Jen Lai[2], Nai-Wen Guo[3], George H. Lambert[4], Yueliang Leon Guo[5]

[1]Department of Safety, Health and Environmental Engineering, National Kaohsiung First University of Science and Technology, Kaohsiung, Taiwan,
[2]Department of Psychiatry, Chung-Shan Medical University Hospital, Taichung, Taiwan,
[3]Department of Psychology, Kaohsiung Medical University, Kaohsiung, Taiwan,
[4]NTH/USEPA Center for Childhood Neurotoxicology and Exposure Assessment and the Department of Pediatrics, University of Medicine and Dentistry of New Jersey and Rutgers University, One Robert Wood Johnson Place, New Brunswick, NJ, USA, and
[5]Department of Environmental and Occupational Health, National Cheng Kung University Medical College, Tainan, Taiwan

Polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) are persistent environmental pollutants shown to adversely interact with several functions of the endocrine system. In 1978–1979, over 2000 Taiwanese people ingested rice oil accidentally contaminated with PCBs and PCDFs. This is one of the major toxic exposure episodes that occurred globally and was later called Yucheng (oil disease in Chinese). The children born to exposed Yucheng women were therefore exposed in utero to high doses of PCBs/PCDFs. In 1995, 60 Yucheng and 61 control boys participated in physical examination, and serum hormones were measured by radioimmunoassay (RIA). Age, body weight, body height, Tanner status, testicular size, serum luteinizing hormone (LH), prolactin (PRL), thyroxine (T_4), triiodothyronine (T_3), and thyroid-stimulating hormone (TSH) levels were not statistically different between Yucheng and control boys in the subgroups of before and at the age of puberty. However, the serum estradiol (E_2) levels were significant higher in Yucheng boys at the age of puberty. Yucheng and control boys were further divided into two subgroups, those before (age <13 yr) and those at the age of puberty (age ≥ 13 yr). There was a decrease of serum testosterone (TT) levels and increase of serum follicle-stimulating hormone (FSH) levels in Yucheng boys at the age of puberty as compared with controls. There was a significant decrease of the square root of TT/E_2 and TT/FSH; however, the square root of E_2/FSH was increased in Yucheng boys at the age of puberty as compared with controls. Data indicated that prenatal exposure to PCBs and PCDFs may have implications for boys' sex hormone homeostasis at puberty. Further studies are needed to identify the congeners of PCBs/PCDFs responsible for disruption of the endocrine system, as well as the mechanisms of such disruption.

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Address correspondence to Dr. Yueliang Leon Guo, Department of Environmental and Occupational Health, National Cheng Kung University, Medical College, 61 Shiao Tong Road, Tainan, 70428, Taiwan. E-mail: leonguo@mail.ncku.edu.tw

Polychlorinated biphenyls (PCBs) and dibenzofurans (PCDFs) are persistent environmental pollutants that induce a broad spectrum of toxic effects in mammalian species (Safe, 1986; DeRosa et al., 1998). PCBs and PCDFs have been produced in large quantities after World War II for industrial uses because of their physical and chemical properties, such as thermal stability, a high dielectric constant, and low volatility. Currently, the use of these substances is restricted in many countries. Their high lipophilicity and resistance to biodegradation has resulted in bioaccumulation in the food chain and led to detectable amounts of PCBs and PCDFs in the environment (Olafsson et al., 1987), human milk (Safe et al., 1985; Diehl-Jones & Bols, 2000) and adipose tissue (Dewailly et al., 1991). Since these substances interact with several functions of the endocrine system, they have been called endocrine disruptors (Kavlock et al., 1996; DeRosa et al., 1998).

Exposure to endocrine disruptors is of particular significance during development, where the fetal testes are regarded as highly sensitive to alterations in hormone levels, especially the balance between estrogen and androgen (Toppari et al., 1996; Jorgensen et al., 1999). Animal studies suggested that prenatal exposure to endocrine disruptors might produce adverse effects on male pubertal development via the changes of sex hormone levels (Wood et al., 1991; Delemarre-van de Waal, 1993; Howdeshell et al., 1999). The effects of an imbalance between estrogen and androgen may induce developmental abnormalities, such as hypospadia and cryptorchidism, oligospermia, and testicular cancer (Jensen et al., 1995; Toppari et al., 1996; Jorgensen et al., 1999). However, serum hormone levels were not significantly affected at the age of puberty in boys prenatally exposed to PCBs in Denmark (Mol et al., 2002).

Over 2000 Taiwanese people in 1978–1979 ingested rice oil accidentally contaminated with PCBs and their heat-degradation products, mainly PCDFs. This is one of the major exposure episodes that occurred in Taiwan (Yucheng, oil disease in Chinese). People developed chloracne, hyperpigmentation, peripheral neuropathy, and other signs and symptoms (Hsu et al., 1985). The Yucheng children were born between 1979 and 1985 to women who had been exposed to high doses of PCBs/PCDFs. Children of these mothers were born growth-retarded, with dysmorphic physical features, delayed cognitive development, behavioral problems, and middle-ear disease (Guo et al., 1994, 1995; Lai et al., 1994; Yu et al., 1994, 1998; Chao et al., 1997). By semen analysis, young men exposed in utero were found to have significantly increased sperm morphological abnormalities and decreased motility, velocity, and capability of oocyte penetration (Guo et al., 2000). However, epidemiological information concerning the effects of PCBs/PCDFs prenatal exposure on male hormone homeostasis is still limited. In this study, experiments were carried out on the examinations of physical development and sex and thyroid hormone levels of the cohort to determine whether prenatal exposure to high levels of PCBs/PCDFs affects hormone homeostasis in boys before and at the age of puberty.

METHODS

Cohort Subjects

The Yucheng registry and the mortality follow-up are described elsewhere (Yu et al., 1997). Briefly, the Yucheng children were born between 1979 and 1985 to women who had been exposed to high doses of PCBs/PCDFs through consumption of contaminated rice bran oil in 1978–1979, and the information was registered by the Taiwan Provincial Department of Health based on signs and symptoms of the illness or the history of the consumption of the concentration of the contaminated oil (Hsu et al., 1985). In 1995, Yucheng boys and their matched control were invited for a complete physical examination. For the follow-up study, an unexposed child was selected as a control for each Yucheng boy, matched for neighborhood (same township), age, gender, mother's age (within 3 yr), parents' combined educational level, and occupation. In total, 105 Yucheng and 101 control boys were invited, and 60 and 61 participated for examination, respectively. Yucheng and control boys were further divided into two subgroups, those before (age < 13 yr) and at the age of puberty (age ≥ 13 yr).

Physical Examinations

The study protocol was approved by the Institution Review Board at National Cheng Kung University Medical College, and complied with the principles outlined in the Declaration of Helsinki (41st World Medical Assembly). Each boy's degree of sexual maturation was assessed by physical examination and rated with a Tanner score from 1 (prepubertal) to 5 (sexually mature) according to their physical secondary sexual characteristics using a slightly modified version of the criteria described by Tanner (van Wieringen et al., 1971). Testicular size and pattern of public hair were assessed by a physician who was not aware of the boy's exposure status. Testicular volume was measured by manual palpation and compared to a standard-sized orchidometer (Prader, 1966).

Serum Hormone Analyses

A total of 96 blood samples were taken from 47 Yucheng boys and 49 control boys by standard puncture of a cubital vein. Blood samples were centrifuged shortly after sampling, and serum was stored at –80°C until analysis. The blood samples were obtained and assessed in 1997 to measure levels of serum testosterone (TT), estradiol (E_2), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroxine (T_4), triiodothyronine (T_3), and thyroid-stimulating hormone (TSH). The concentrations of serum hormones were measured by radioimmunoassay (RIA) methods (Wiersinga & Chopra, 1982) at the central laboratory of National Cheng Kung University Hospital; laboratory personnel were unaware of the PCBs/PCDFs exposure status of the subjects. All the standard assay kits (International CIS radioimmunoassay kits, USA) were based on the general principle of the law of mass

action between the analyte and the specific antibody on the one hand and their interaction product on the other. The added ^{125}I-labeled and the unlabeled antibody react at a similar rate with the analyte, so that the separation and counting of the bound fraction allow the analyte to be quantified by using the calibration curve. Each sample was assayed at one or two dilutions in duplicate. The intra- and interassay variation coefficients of the internal quality control pools were all within 10%.

Statistical Analysis

All interview, examinations, and laboratory tests including hormone analyses were done in a blinded fashion. All the values are presented as means ± SD. Comparisons of age, body weight, body height, Tanner status, testicular size, sex hormone levels, and thyroid hormone levels between Yucheng and control boys before and at the age of puberty were done by Student's *t*-test using a JMP statistical package (SAS Institute, Inc., Cary, NC). Hormone values were transformed according to their original distribution into normal distribution; for example, testosterone/estradiol values were transformed into square roots. The confidence limit was set at $p < .05$.

RESULTS

Physical Status and Sexual Differentiation

Sixty Yucheng boys and 61 control boys volunteered to participate the physical examination. They were grouped according to age into prepubertal (< 13 yr) and pubertal (≥13 yr) groups. Age, body weight, body height, Tanner status, and right and left testicular size were not statistically different between Yucheng and control boys in the subgroups (Table 1).

Sex Hormone Levels

The serum E_2 levels were significantly higher in Yucheng boys at the age of puberty ($p = .052$) (Table 2). There was no marked change in serum TT levels in Yucheng boys at the age of puberty as compared with controls. In addition, no significant difference was found in serum FSH levels in Yucheng boys at the age of puberty (Table 2). Moreover, the correlations between TT, E_2, and FSH showed a significant decrease of the square root of TT/E_2 ($p = .017$) in Yucheng boys at the age of puberty as compared with their controls. Levels of TT, E_2, FSH, square root of TT/E_2, TT/FSH, and E_2/FSH were not statistically different between Yucheng and control boys aged younger than 13 yr (Table 2). Serum LH and PRL levels were also not statistically different between Yucheng and control boys in those younger and older than 13 yr of age (Table 2).

Thyroid Hormone Levels

For T_4, T_3, and TSH, the mean concentrations were within the range of normal values (for T_4, from 4 to 12 μg/dl; for T_3, from 50 to 150 ng/dl; for TSH,

TABLE 1. Age and Physical Examination in Boys Prenatally Exposed to PCBs/PCDFs (Yucheng) and Unexposed Controls in the Subgroups of All Ages Before and at the Age of Puberty

Category	Control	Yucheng
Age (Yr)		
All age	12.3 ± 1.9 (*n* = 61)	12.3 ± 1.9 (*n* = 60)
Before puberty[a] (age <13 yr)	11.1 ± 0.2 (*n* = 40)	11.2 ± 0.2 (*n* = 41)
Puberty[b] (age ≥13 yr)	14.5 ± 1.0 (*n* = 21)	14.6 ± 1.0 (*n* = 19)
Body weight (kg)		
All age	42.6 ± 13.1 (*n* = 48)	42.4 ± 12.9 (*n* = 49)
Before puberty[a] (age <13 yr)	37.3 ± 12.2 (*n* = 31)	36.6 ± 10.4 (*n* = 34)
Puberty[b] (age ≥13 yr)	52.1 ± 8.6 (*n* = 17)	55.6 ± 7.3 (*n* = 15)
Body height (cm)		
All age	150.1 ± 13.2 (*n* = 48)	148.6 ± 13.6 (*n* = 49)
Before puberty[a] (age <13 yr)	143.0 ± 9.5 (*n* = 31)	142.1 ± 10.3 (*n* = 34)
Puberty[b] (age ≥13 yr)	164.1 ± 4.7 (*n* = 17)	163.4 ± 6.6 (*n* = 15)
Tanner status		
All age	2.1 ± 1.3 (*n* = 47)	2.0 ± 1.2 (*n* = 48)
Before puberty[a] (age <13 yr)	1.4 ± 0.8 (*n* = 30)	1.5 ± 0.8 (*n* = 33)
Puberty[b] (age ≥13 yr)	3.3 ± 1.2 (*n* = 17)	3.2 ± 0.9 (*n* = 15)
Right testicular size		
All age	9.3 ± 7.4 (*n* = 47)	8.3 ± 7.0 (*n* = 47)
Before puberty[a] (age <13 yr)	5.0 ± 4.3 (*n* = 30)	5.2 ± 4.0 (*n* = 33)
Puberty[b] (age ≥13 yr)	16.9 ± 5.3 (*n* = 17)	15.7 ± 7.2 (*n* = 14)
Left testicular size		
All age	9.8 ± 8.0 (*n* = 47)	8.9 ± 7.3 (*n* = 47)
Before puberty[a] (age <13 yr)	5.1 ± 4.5 (*n* = 30)	5.2 ± 4.0 (*n* = 33)
Puberty[b] (age ≥13 yr)	18.1 ± 5.6 (*n* = 17)	17.8 ± 5.5 (*n* = 14)

[a]Before the age of puberty: Their mothers were pregnant after 1981 with duration of exposure to PCBs/PCDFs large than 2 yr.

[b]At the age of puberty: Their mother were pregnant before 1981 with duration of exposure to PCBs/PCDFs less than 2 yr.

from 0.3 to 5 mU/ml), and all measurements were above the detection limit for the assay. No significant differences in the concentrations of T_4, T_3, square root of T_3/T_4, and TSH were observed between Yucheng and control boys in both age subgroups (Table 3).

DISCUSSION

The Yucheng cohort represents one of the largest groups of children prenatally exposed to PCBs/PCDFs. Even 13 yr after the episode, the chemicals persisted in mothers' bodies (Guo et al., 1997) and the Yucheng children continued to demonstrate adverse effects from their mothers' exposure, although it had been almost two decades since the Yucheng outbreak (Guo et al., 2004). In this study, Yucheng and control boys were divided into two subgroups according to their age, before puberty (<13 yr) or at puberty (≥13 yr).

TABLE 2. Levels of Sex Hormones for Prenatal PCB/PCDF-Exposed (Yucheng) Boys and Unexposed Controls in the Subgroups of All Ages Before and at the Age of Puberty

Sex hormones	Control	Yucheng
Testosterone (TT, ng/ml)		
All age	2.2 ± 2.4 (n = 47)	1.8 ± 2.1 (n = 49)
Before puberty[a] (age <13 yr)	1.0 ± 1.5 (n = 30)	1.3 ± 1.6 (n = 34)
Puberty[b] (age ≥13 yr)	4.2 ± 2.2 (n = 17)	3.0 ± 2.4 (n = 15)
Estradiol (E_2, pg/ml)		
All age	14.9 ± 11.1 (n = 45)	23.7 ± 35.7 (n = 49)
Before puberty[a] (age <13 yr)	11.0 ± 7.6 (n = 28)	12.6 ± 14.6 (n = 34)
Puberty[b] (age ≥13 yr)	21.3 ± 13.2 (n = 17)	48.6 ± 53.9 (n = 15)[c]
$\sqrt{(TT/E_2)}$		
All age	0.4 ± 0.2 (n = 45)	0.3 ± 0.2 (n = 49)
Before puberty[a] (age <13 yr)	0.3 ± 0.2 (n = 28)	0.3 ± 0.2 (n = 34)
Puberty[b] (age ≥13 yr)	0.5 ± 0.2 (n = 17)	0.3 ± 0.2 (n = 15)[d]
Follicle-stimulating hormone (FSH, mIU/ml)		
All age	3.4 ± 1.6 (n = 45)	3.6 ± 1.8 (n = 49)
Before puberty[a] (age <13 yr)	3.3 ± 2.0 (n = 28)	3.3 ± 1.5 (n = 34)
Puberty[b] (age ≥13 yr)	3.4 ± 0.8 (n = 17)	4.6 ± 2.2 (n = 15)
$\sqrt{(TT/FSH)}$		
All age	0.5 ± 0.4 (n = 45)	0.6 ± 0.4 (n = 49)
Before puberty[a] (age <13 yr)	0.5 ± 0.4 (n = 28)	0.8 ± 0.5 (n = 34)
Puberty[b] (age ≥13 yr)	1.1 ± 0.3 (n = 17)	0.8 ± 0.5 (n = 15)
$\sqrt{(E_2/FSH)}$		
All age	2.2 ± 1.1 (n = 45)	2.3 ± 1.2 (n = 49)
Before puberty[a] (age <13 yr)	2.0 ± 1.2 (n = 28)	2.0 ± 0.9 (n = 34)
Puberty[b] (age ≥13 yr)	2.4 ± 0.8 (n = 17)	3.1 ± 1.4 (n = 15)
Luteinizing hormone (LH, mIU/ml)		
All age	1.8 ± 1.2 (n = 45)	1.9 ± 1.2 (n = 49)
Before puberty[a] (age <13 yr)	1.5 ± 1.2 (n = 28)	1.5 ± 1.0 (n = 34)
Puberty[b] (age ≥13 yr)	2.4 ± 1.0 (n = 17)	2.6 ± 1.5 (n = 15)
Prolactin (PRL, ng/ml)		
All age	0.3 ± 0.3 (n = 45)	0.4 ± 0.3 (n = 49)
Before puberty[a] (age <13 yr)	0.4 ± 0.3 (n = 28)	0.4 ± 0.4 (n = 34)
Puberty[b] (age ≥13 yr)	0.3 ± 0.1 (n = 17)	0.4 ± 0.3 (n = 15)

Note. Limit of detection: TT, 0.04 ng/ml; E_2, 8 pg/ml; FSH, 0.06 mIU/ml; LH, 0.15 mIU/ml; PRL, 0.1 ng/ml.

[a]Before the age of puberty: Their mothers were pregnant after 1981 with duration of exposure to PCBs/PCDFs large than 2 yr.

[b]At the age of puberty: Their mothers were pregnant before 1981 with duration of exposure to PCBs/PCDFs less than 2 yr.

[c]Significant, $.05 < p < .1$.

[d]Significant, $0 < p < .05$.

Testosterone, estradiol, and LH levels were lower before puberty, and higher after the age of 13 yr. The other hormones were not changed across time of pubertal development. Levels of serum hormones before puberty were not

TABLE 3. Levels of Thyroid Hormones for Prenatal PCB/PCDF-Exposed (Yucheng) Boys and Unexposed Controls in the Subgroups of All Ages Before and at the Age of Puberty

Thyroid hormones	Control	Yucheng
Thyroxine (T_4, μg/dl)		
All age	8.9 ± 1.8 (n = 46)	9.3 ± 1.9 (n = 49)
Before puberty[a] (age <13 yr)	9.1 ± 1.8 (n = 29)	9.6 ± 1.9 (n = 34)
Puberty[b] (age ≥13 yr)	8.5 ± 1.6 (n = 17)	8.6 ± 1.7 (n = 15)
Triiodothronine (T_3, ng/dl)		
All age	121.5 ± 31.1 (n = 46)	131.5 ± 32.3 (n = 49)
Before puberty[a] (age <13 yr)	123.7 ± 33.9 (n = 29)	135.1 ± 34.5 (n = 34)
Puberty[b] (age ≥13 yr)	117.8 ± 26.1 (n = 17)	123.4 ± 25.9 (n = 15)
$\sqrt{(T_3.T_4)}$		
All age	3.7 ± 0.3 (n = 45)	3.8 ± 0.3 (n = 49)
Before puberty[a] (age <13 yr)	3.7 ± 0.3 (n = 28)	3.7 ± 0.3 (n = 34)
Puberty[b] (age ≥13 yr)	3.7 ± 0.3 (n = 17)	3.8 ± 0.3 (n = 15)
Thyroid-stimulating hormone (TSH, mIU/L)		
All age	2.0 ± 1.1 (n = 46)	2.1 ± 1.4 (n = 49)
Before puberty[a] (age <13 yr)	2.1 ± 1.2 (n = 29)	2.3 ± 1.5 (n = 33)
Puberty[b] (age ≥13 yr)	1.8 ± 1.1 (n = 17)	1.8 ± 1.1 (n = 15)

Note. Limit of detection: T_4, 0.25 μg/dl; T_3, 7 ng/dl; TSH, 0.1mIU/L.

[a] Before the age of puberty: Their mothers were pregnant after 1981 with duration of exposure to PCBs/PCDFs longer than 2 yr.

[b] At the age of puberty: Their mothers were pregnant before 1981 with duration of exposure to PCBs/PCDFs less than 2 yr.

different between Yucheng and control boys. However, serum E_2 and FSH levels were significant higher, and square root of TT/E_2 was significantly decreased in Yucheng boys 13 yr or older.

In normal adult men, the testes and adrenals are the major source of androgens; most estrone and estradiol are derived secondarily from the aromatization of androstenedione and testosterone in peripheral tissues (Longcope et al., 1969; Vermeulen et al., 1972). In Yucheng boys, the right and left testicular size was not affected. However, the ratio of TT and E_2 was significantly decreased at puberty. By semen analysis, Yucheng young men 16–20 yr of age were found to have sperm with increased morphological abnormalities and decreases of motility, velocity, and capability of hamster oocyte penetration (Guo et al., 2000). A possible mechanism of decreased spermatogenesis is a decrease in FSH and/or TT levels. The homeostasis between FSH and TT is important for quantitatively normal spermatogenesis (Steinberger & Steinberger, 1989). Homeostasis could be disrupted by xenobiotic-induced alterations of sex steriod metabolizing cytochrome P450 enzymes. The natural balance of circulating sex hormones could conceivably be changed from normal by inducing or blocking these enzymes (Parks & LeBlanc, 1998; Wilson et al., 1999; Wilson & LeBlanc, 2000). In contaminated lakes of Florida, male juvenile alligators prenatally exposed to pesticide were found to have significantly

depressed plasma TT levels, poorly organized testes, and abnormally small phalli (Guillette et al., 1994), as well as changed sexually dimorphic biotranformation of TT (Gunderson et al., 2001). Therefore, pollutant chemicals that are widespread in the environment can affect endocrine signaling, as evidenced in laboratory experiments and in wildlife with relatively high exposures. Although humans are commonly exposed to such pollutant chemicals, the exposure levels are generally low, and clear effects on endocrine function from such exposures have been difficult to demonstrate (Rogan & Ragan, 2003).

In this study, no significant differences were found in the serum concentration of T_4, T_3, TSH, and T_3/T_4, ratios between exposed and control boys. A study in the Netherlands on 38 healthy term breast-fed infants found higher neonatal serum T_4 concentrations and elevated T_4/TBG ratios in the highly exposed group (Pluim et al., 1992, 1993). A study of a similar group of 78 Dutch children found lower serum free T_4 levels and higher TSH levels in the highly exposed group of neonates compared with the low-exposure group (Koopman-Esseboom et al., 1994). In the neonates, free T_4 and TSH concentrations were not related to serum PCB levels in the two Dutch studies. In Slovakia, Langer et al. (1998) found increased prevalence of thyroid disorder in the employees of a factory where PCBs had been produced previously and adolescents in the surrounding area had been exposed to PCBs. These effects presumably resulted from long-term direct effects of organochlorines on thyroid structure, interference with peripheral thyroid hormone metabolism, and immunomodulatory and other nonspecific effects as suggested by Li and Hansen (1997). In the United States, in 160 children from central North Carolina with in utero exposure to background levels of PCBs, cord serum thyroid hormone and TSH levels were not correlated with exposure levels (Longnecker et al., 2000).

Our results indicated that maternal exposure to PCBs/PCDFs may have implications for young men's sex hormone homeostasis at puberty. Whether this effect is induced by residual levels of contaminants in the boys or by the changed hormone homeostasis due to damage in utero is unclear. The Yucheng subjects were exposed to a complex mixture of PCBs and PCDFs. Further studies are warranted to look into which PCB/PCDF congeners are involved in the disruption of endocrine system, and to determine the biological mechanisms underlying this effect.

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